

PATENT

Attorney Docket No.: 44805-0001 DI1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Patent application of  
Jeffrey D. Brady  
Simon P. Robins

Serial No.: Not Yet Assigned

Filed: Herewith

For: METHOD OF ASSAYING  
PYRROLE-CONTAINING  
BIOLOGICAL COMPOUNDS

Group Art Unit:  
Not Yet Assigned


Examiner:  
Not Yet Assigned

MAIL STOP PATENT APPLICATION  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**INFORMATION DISCLOSURE STATEMENT**

Sir:

Pursuant to 37 C.F.R. § 1.56 and in accordance with 37 C.F.R. §§1.97-1.98,  
submitted herewith is an accompanying substitute Form PTO-1449.

<b>CERTIFICATE OF MAILING</b> UNDER 37 C.F.R. 1.10
EXPRESS MAIL Mailing Label Number: EV320479062US Date of Deposit: <u>September 26, 2003</u>
I hereby certify that this correspondence, along with any paper referred to as being attached or enclosed, and/or fee, is being deposited with the United States Postal Service, "EXPRESS MAIL-POST OFFICE TO ADDRESSEE" service under 37 CFR 1.10, on the date indicated above, and addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.
 Signature of person mailing page: <u>KAREN M. SPINA</u> Type or print name of person

The Examiner is requested to review each of the references and make them of record during the prosecution of this application as required by M.P.E.P. §609. It is requested that the Examiner initial the duplicate substitute Form 1449, and return one copy to the undersigned.

Pursuant to 37 C.F.R. § 1.98(d), copies of the references listed in the PTO Form 1449 are not enclosed, since this patent application is a divisional of U.S. Application No. 09/970,328, filed October 3, 2001. A copy of each listed reference is contained in the file of Application No. 09/970,328. The Examiner should contact the undersigned if additional copies of any of the listed references are needed.

A photocopy of the Information Disclosure Statement filed January 16, 2003 in U.S. Application No. 09/970,328 is submitted herewith, providing a brief characterization of the references, except for references DA and DB.

This Information Disclosure Statement should not be construed as a representation that the cited references are material or that no better art exists.

Respectfully submitted,  
JEFFREY D. BRADY, et al.



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Attorney for the Applicants

SUBSTITUTE FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE  <b>INFORMATION DISCLOSURE CITATION</b>	ATTY. DOCKET NO. <b>44805-0001 D11</b>	SERIAL NO. not yet assigned
	APPLICANT: <b>Jeffrey D. Brady, et al</b>	
	FILING DATE <b>Herewith</b>	GROUP

## U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)
	AA	5,532,169	7/2/96	Eyre	436	518	
	AB	5,641,837	6/24/97	Eyre	435	7.1	
	AC	5,652,112	7/29/97	Eyre	435	7.1	
	AD	5,834,221	11/10/98	Eyre	435	7.92	
	AE	5,576,189	11/19/96	Eyre	435	7.93	
	AF	5,656,439	8/12/97	Eyre	435	7.1	
	AG	5,945,274	8/31/99	Eyre	435	4	
	AH	4,973,666	11/27/90	Eyre	530	323	
	AI	5,939,274	8/17/99	Eyre	435	7.1	
	AJ	5,736,344	4/7/98	Kung	435	7.9	
	AK	5,972,623	10/26/99	Krano	435	7.1	
	AL	6,010,863	1/4/00	Te Koppele	435	7.1	
	AM	6,025,144	2/15/00	Eyre	435	7.1	
	AN	6,027,903	2/22/00	Eyre	435	7.1	
	AO	5,962,236	10/5/99	Eyre	435	7.1	
	AP	5,641,687	6/24/97	Eyre	436	518	
	AQ	5,455,179	10/3/95	Eyre	436	536	
	AR	5,919,634	7/6/99	Eyre	435	7.1	
	AS	5,702,909	12/30/97	Eyre	435	7.9	
	AT	5,300,434	4/5/94	Eyre	435	240.2	
	AU	5,473,052	12/5/95	Eyre	530	387.9	
	AV	5,700,694	12/23/97	Robins	436	64	
	AW	4,628,027	12/9/86	Gay	435	7	
	AX	5,912,131	6/15/99	Eyre	435	7.1	
	AY	5,472,884	12/5/95	Eyre	436	518	
	AZ	5,320,970	6/14/94	Eyre	436	536	
	BA	5,962,639	10/5/99	Eyre	530	329	
	BB	5,140,103	8/18/92	Eyre	530	327	
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	BD	5,817,755	10/6/98	Eyre	530	328	
	BE	5,700,693	12/23/97	Robins	436	64	
	BF	5,677,198	10/14/97	Eyre	436	518	
	BG	5,607,862	3/4/97	Eyre	436	501	
	BH	5,688,652	11/18/97	Eyre	435	7.1	

## FOREIGN PATENT DOCUMENTS

		DOCUMENT NO.	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
	BI	WO86/06374	11/6/86	PCT	C07D	309/34	YES	NO

## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

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EXAMINER	DATE CONSIDERED
EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

SUBSTITUTE FORM PTO-1449  U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE  <b>INFORMATION DISCLOSURE CITATION</b>	ATTY. DOCKET NO. <b>44805-0001 D11</b>	SERIAL NO. <b>Not yet assigned</b>
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## FOREIGN PATENT DOCUMENTS

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## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, Etc.)

CA	Adamczyk, et al.: "Synthesis of Immunoreagents for Detection of Deoxypyrrroline, a Cross-link of Bone Collagen", <i>Bioorganic &amp; Medicinal Chemistry Letters</i> , Vol. 10, pp. 269-271 (2000)
CB	Atley, et al.: "Proteolysis of Human Bone Collagen by Cathepsin K: Characterization of the Cleavage Sites Generating the Cross-Linked N-Telopeptide Neopeptide", <i>Bone</i> , Vol. 26, No. 3, pp. 241-247, March 2000
CC	Brame, et al: Identification of Extremely Reactive $\gamma$ -Ketoaldehydes (Isolevuglandins) as Products of the Isoprostane Pathway and Characterization of Their Lysyl Protein Adducts", <i>The Journal of Biological Chemistry</i> , Vol. 274, No. 19, pp. 13139-13146, May 7, 1999
CD	Hanson, et al: "Molecular Site Specificity of Pyridinoline and Pyrrole Cross-links in Type I Collagen of Human Bone", <i>The Journal of Biological Chemistry</i> , Vol. 271, No. 43, pp. 26508-26516, October 25, 1996
CE	Hughes, et al: "A Collagen-associated Ehrlich Chromogen: a Pyrrolic Cross-link?", <i>Bioscience Reports (UK)</i> , Vol. 1, pp. 611-618, (1981)
CF	Kemp, et al: "Ehrlich Chromogens, Probable Cross-links in Elastin and Collagen", <i>Biochemical Journal (UK)</i> , Vol. 252, pp. 387-393 (1988)
CG	Kuypers, et al: "Identification of the Loci of the Collagen-associated Ehrlich Chromogen in Type I Collagen Confirms its Role as a Trivalent Cross-link", <i>Biochemical Journal (UK)</i> , Vol. 283, pp. 129-136 (1992)
CH	Lombard, et al: "Comparison of Three Reagents for Detecting Indole Production by Anaerobic Bacteria in Microtest Systems", <i>Journal of Clinical Microbiology</i> , Vol. 18, No. 3, pp. 609-613, September 1983
CI	McBrayer, et al: "Diffusion of Metals in Silicon Dioxide", <i>Journal of Electrochemical Society</i> , Vol. 133, No. 6, pp. 1242-1246, June 1986
CJ	Raghavan, et al: "Diffusion of Copper Through Dielectric Films Under Bias Temperature Stress", <i>Thin Solid Films</i> , Vol 262, pp. 168-176 (1995)
CK	Rajkumar, et al: "Generation of Pyrroles in the Reaction of Levuglandin E <sub>2</sub> with Proteins", <i>Journal of Organic Chemistry</i> , Vol. 59, pp. 6038-6043, (1994)
CL	Ramanakoppa H. Nagaraj, et al: "Protein Modification by the Degradation Products of Ascorbate: Formation of a Novel Pyrrole from the Maillard Reaction of L-threose with Proteins", <i>Biochimica et Biophysica Acta</i> , Vol. 1253, pp. 75-84 (1995)
CM	Salomon, et al: "Protein Adducts of Iso[4]levuglandin E <sub>2</sub> , a Product of the Isoprostane Pathway, in Oxidized Low Density Lipoprotein", <i>The Journal of Biological Chemistry</i> , Vol. 274, No. 29, pp. 20271-20280, July 16, 1999
CN	Scott, et al: "An 'Affinity' Method for Preparing Polypeptides Enriched in the Collagen-Associated Ehrlich Chromogen", <i>Journal of Biochemistry</i> , Vol. 93, pp. 921-925 (1983)
CO	Chemical Abstracts 130:53792 abstracting German patent application 19723779 (1997)
CP	Chemical Abstracts 127:122001 abstracting PCT Publication No. WO 9721685 (1997)
CQ	Chemical Abstracts 126:343579 abstracting PCT Publication No. WO 9714685 (1997)
CR	Chemical Abstracts 128:47979 abstracting Japanese Publication No. 09301939 (1997)
CS	Chemical Abstracts 125:86316 abstracting PCT Publication No. WO 9608483 (1996)
CT	Chemical Abstracts 125:181171 abstracting European Patent No. 718710 (1996)
CU	Chemical Abstracts 125:181170 abstracting European Patent No. 718709 (1996)
CV	Chemical Abstracts 125:154318 abstracting European Patent No. 718708 (1996)
CW	Chemical Abstracts 124:261609 abstracting European Publication No. 689845 (1996)
CX	Chemical Abstracts 121:10000 abstracting German Publication No. 4232505 (1994)
CY	Chemical Abstracts 120:271175 abstracting PCT Publication No. 9315047 (1993)
CZ	Chemical Abstracts 83:44737 abstracting German Publication No. 2435888 (1975)
DA	G.K. Reddy and C.S. Enwemeka: "Method for Assaying Pyrrole-Containing biological Compounds", <i>Clinical biochemistry</i> 29(3): 225-229 (1996)
DB	M. Stefek, A. Gajdosik, a. Gajdosikova, L. Krizanova, "Dimethylaminobenzaldehyde-reactive substances in tail tendon collagen of streptozotocin-diabetic rats: temporal relation to biomechanical properties and advanced glycation endproduct (AGE)-related fluorescence", <i>Biochimica et Biophysica Acta</i> 1502:398-404 (2000)

EXAMINER	DATE CONSIDERED
EXAMINER: Initial if citation considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

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PATENT

Attorney Docket No.: 8830-6 CI 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Patent application of  
Jeffrey D. Brady, et al

Group Art Unit: 1641

Serial No.: 09/970,328

Filed: October 3, 2001

Examiner: Not yet  
assigned

For: METHOD OF ASSAYING  
PYRROLE-CONTAINING BIOLOGICAL  
COMPOUNDS

INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents  
Washington, D.C. 20231

Sir:  
Pursuant to 37 C.F.R. § 1.56 and in accordance with 37 C.F.R. §§1.97-1.98,  
submitted herewith are copies of the references listed in the accompanying Form PTO-1449.

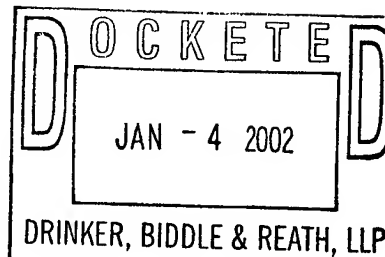
The examiner is requested to review the items listed on the attached PTO  
Form-1449 and make them of record in the instant application as required by M.P.E.P. §609.  
It is requested that the Examiner initial the enclosed duplicate substitute Form -1449, and  
return one copy to the undersigned.

CERTIFICATE OF MAILING  
UNDER 37 C.F.R. 1.8(a)

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DATE: 1/3/01

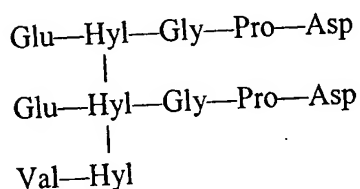


This Information Disclosure Statement should not be construed as a representation that the cited references are material or that no better art exists.

### **Brief Characterization of References**

#### **US 5,532,169**

A method of determining carilage degradation. The method includes quantitating the concentration of a peptide in a body fluid sample. The peptide comprises:



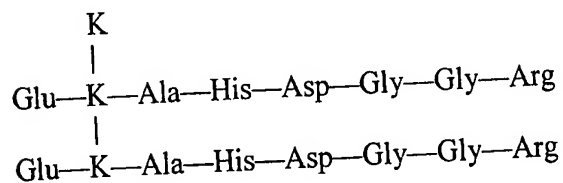
Wherein Hyl – Hyl – Hyl is hydroxylysyl pyridinoline.

#### **US 5,641,837**

A method of determining cartilage degradation. The method includes analysing a body fluid sample for the presence of an analyte by assessing the binding of the analyte to an immunological binding partner. The immunological binding partner is capable of binding to a peptide comprising a c-terminal type II collagen telopeptide containing a hydroxylysyl pyridinoline cross-link.

#### **US 5,652,112**

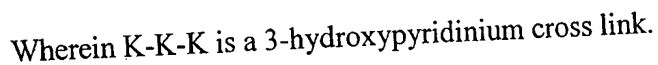
A method of analysing a body fluid sample for the presence of an anlyte indicative of type I collagen degradation. The method includes assessing the binding of the analyte to an immunological binding partner. The immunological binding partner is capable of binding to a peptide containing a 3-hydroxypyridinium cross-link derived from the carboxyterminal telopeptide domain of type I collagen. The peptide comprises:



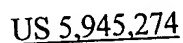
Wherein K-K-K is a 3-hydroxypyridinium cross-link selected from hydroxylysyl pyridinoline and lysyl pyridinoline.

A method of analysing a body fluid sample for the presence of an analyte indicative of type I collagen degradation. The method includes assessing the binding of the analyte to an immunological binding partner. The immunological binding partner is capable of binding to a peptide derived from type I collagen. The peptide comprises two amino acid sequences derived from the carboxy-terminal telopeptide domain of the X1 (I) chain of type I collagen.

A method of analysing a body fluid sample for the presence of an analyte indicative of a type I collagen degradation. The method includes assessing the binding of the assay to an immunological binding partner. The immunological binding partner is capable of binding to a peptide comprising:



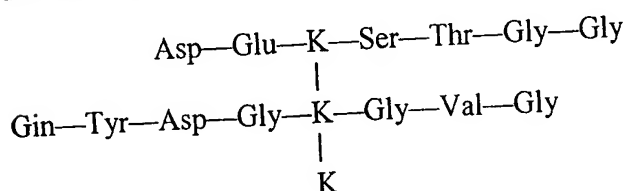
A method of analysing a body fluid sample for the presence of an analyte indicative of a type I collagen degradation in vivo. The immunological binding partner is capable of binding to a peptide comprising:



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US 4,973,666

A method for assaying bone resorption rates by quantitating the concentration of peptide fragments derived from bone collagen, found in a body fluid. The method includes immunometric assay, fluometric assay and electrochemical titration. The structure of specific peptide fragments having 3-hydroxypyridinium cross-links found in urine of Paget's disease patients and procedures for making monoclonal antibodies is also described, particularly a purified peptide fragment comprising the following amino acid sequence:



US 5,834,221

A method of analysing a body fluid sample for the presence of an analyte indicative of type I collagen degradation. The method includes assessing the binding of the analyte to an immunological binding partner to the analyte. The immunological binding partner is capable of binding to a peptide containing a 3-hydroxypyridinium cross-link derived from type I collagen.

US 5,939,274

A method of monitoring a patient's response to an anti-resorptive therapy. The method includes assessing any binding between a body fluid sample and an immunological binding partner specific for a cross-linked telopeptide having a sequence identical to that of a cross-linked amino-terminal or carboxy-terminal telopeptide produced in vivo upon degradation of type I collagen.

US 5,736,344

A method of assaying bone collagen breakdown levels in a human subject useful to screen for the presence of bone resorption disorders. The method includes assessing any binding between a body fluid sample and an antibody, wherein the antibody is specific to N-Pyd and/or N-Dpd.

US 5,972,623

A method of determining the level of type I collagen fragments in a biological fluid using an antibody which is immunospecific for an epitope contained in one of the following sequences:

1. Ala-Hyp-Gly-Asp-Arg-Gly-Glu-Hyp-Gly-Pro-Hyp-Gly-Pro-Ala, or
2. Gly-Asn-Ser-Gly-Glu-Hyp-Gly-Ala-Hyp.



Under conditions effective to allow determination of the level of collagen fragments in the sample which contain the epitope. The method is useful for assessing the level of bone collagen degradation, particularly in humans. Also disclosed are antibodies and kits which can be used in the method.

**The following Patents relate to immunoassays to determine collagen degradation or for assessing bone resorption.**

US 6,010,863

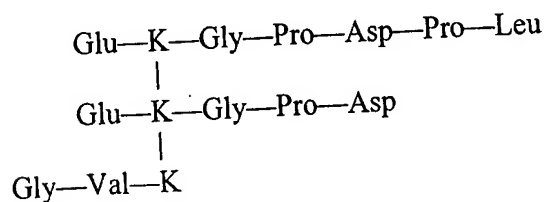
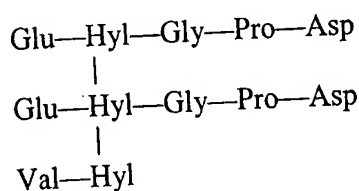
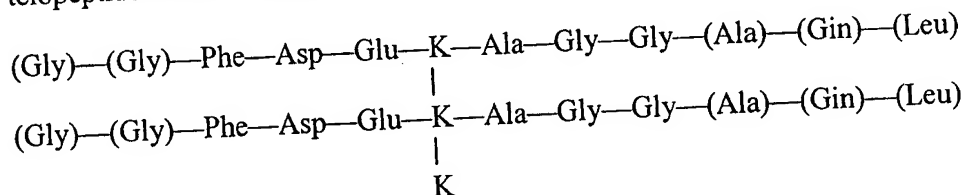
A sandwich-type immunoassay for the detection and/or quantitation of collagen degradation products in biological samples such as blood, serum, plasma, sputum and cell cultures. The immunoassay uses a first antibody directed to an epitope present on a collagen molecule at a distance of up to 165 amino acids from a collagen telopeptide crosslink site, and a second antibody directed at another epitope of the crosslinked collagen molecule.

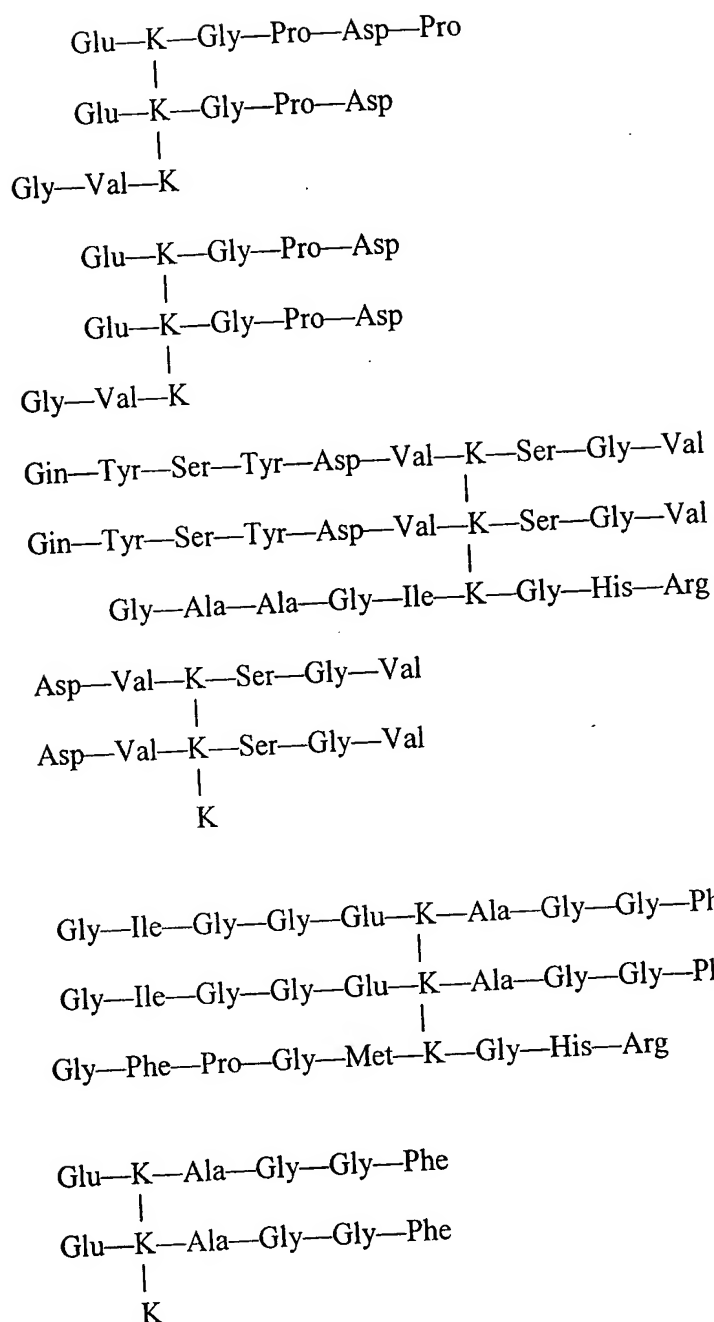
US 6,025,144

An immunoassay test kit including an immunological binding partner that binds to lysyl pyridinoline, for analysing a body fluid sample for bone resorption in vivo.

US 6,027,903

Immunoassay test kit for detecting analyte indicative of type I collagen resorption in vivo, comprising an immunological binding partner which binds to an amino-terminal or carboxy-terminal 3-hydroxypyridinium cross-linked telopeptide of type I collagen isolatable from a urine sample of a patient with active Paget's disease, wherein the immunological binding partner does not cross-react more than 10% with the type II and type III collagen telopeptides of the following formulas:





**The following Patents disclose a method of determining the presence of a physiological condition by assessing the binding of an immunological binding partner to a specific peptide.**

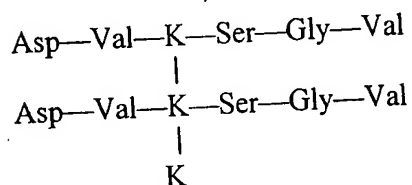
US 5,962,236

A method of analysing a body fluid sample for the presence of an analyte indicative of a physiological condition. The method includes assessing the binding of the analyte to an

immunological binding partner. The immunological binding partner is capable of binding to free lysyl pyridinoline cross-links and the body fluid sample is an unhydrolysed urine sample.

#### US 5,641,687

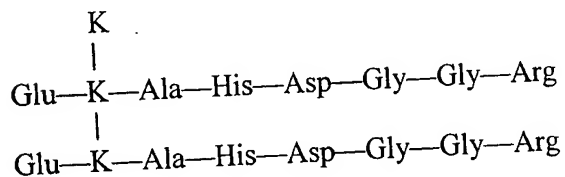
A method of analysing a body fluid sample for the presence of an analyte indicative of a physiological condition. The method includes assessing the binding of the analyte to an immunological binding partner to the analyte. The immunological binding partner is capable of binding to a peptide comprising:



Wherein K-K-K is hydroxylysyl pyridinoline or lysyl pyridinoline.

#### US 5,455,179

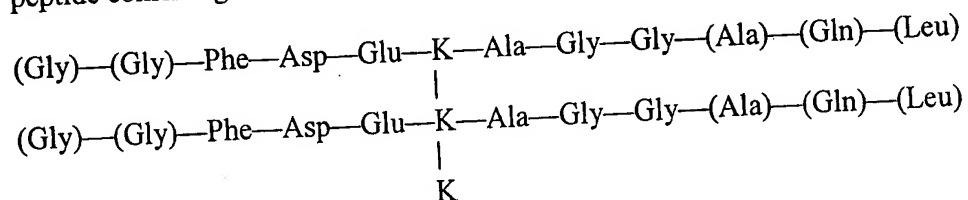
A method of analysing a body fluid sample for the presence of an analyte indicative of a physiological condition. The method includes the step of assessing the binding of an immunological binding partner to the analyte. The immunological binding partner binds to:



Wherein K-K-K is lysyl pyridinoline or hydroxylysyl pyridinoline

#### US 5,919,634

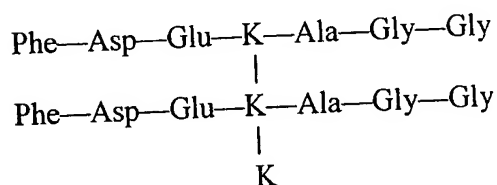
A method of analysing a body fluid sample for the presence of an analyte indicative of a physiological condition. The method includes assessing the binding of the analyte to an immunological binding partner, and correlating any detected binding to the physiological condition. The immunological binding partner is capable of binding to a cross-linked peptide consisting of:



Wherein K-K-K is hydroxylysyl pyridinoline or lysyl pyridinoline. The parentheses indicate optional amino acid residues.

US 5,702,909

A method of analysing a body fluid sample for the presence of an analyte indicative of a physiological condition. The method includes assessing the binding of the analyte to an immunological binding partner to the analyte. The immunological binding partner is capable of binding to a peptide comprising:

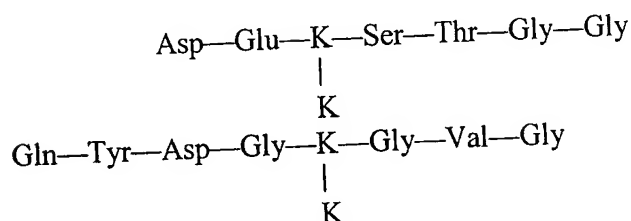


K-K-K is hydroxylysyl pyridinoline or lysyl pyridinoline.

**The following Patents relate to cell lines, or fragments of monoclonal antibodies**

US 5,300,434

A cell line that produces a specific binding partner that binds to first and second peptide consisting essentially of the structure:



Wherein K-K-K is hydroxylysyl pyridinoline or lysyl pyridinoline.

US 5,473,052

An antigen-binding fragment of a monoclonal antibody wherein said antibody is produced by a cell having the identifying characteristics of ATTC No HB 10611 and the antigen-binding fragment is isolated chain of the antibody or is selected from the group consisting of Fab, Facb, F(ab')<sub>2</sub>, Fab' and Fd fragments of the antibody.

**The following Patents relate to methods for assessing connective tissue**

US 5,700,694

A method to assess connective tissue (esp. bone) in order to assess metabolism in disease or to monitor therapy. The method comprises assessing the levels of native free collagen-

derived crosslinks in biological fluids especially urine. The method can be enhanced by concomitantly determining the levels of an indicator of bone formation in biological fluids of the same indiv. and assessing the differences between the degradation marker and the formation indicator. Antibodies which are specifically immunoreactive with forms of crosslinks which occur free in biological fluids are also disclosed.

US 4,628,027

In vitro diagnostic methods using monoclonal antibodies specific for corrective tissue proteins are used to form a collagen profile of human body tissues and fluids. By assessing changes in these profiles, the effectiveness of a treatment, for inflammatory diseases, fibrotic diseases and cancer can be assessed.

**The following Patents relate to specific compositions**

US 5,912,131

A composition comprising peptides produced by digesting bone collagen with a protease capable of generating peptides that bind to Mab 1H11. The digested bone collagen is then purified to increase the concentration of peptides that contain a 3-hydroxypyridinium cross-link by at least 10-fold.

US 5,472,884

Compositions useful in quantitating collagen peptides to determine the rate of bone resorption are prepared by treating bone with a protease, such as collagenase, and purifying the compositions so as to enrich them with peptides capable of binding to the monoclonal antibody Mab-1H11.

US 5,320,970

Compositions useful in quantitating collagen peptides to determine rate of bone resorption are prepared by treating bone with a protease, such as collagenase, and purifying the compositions so as to enrich them with peptides capable of binding to the monoclonal Mab-1H11.

**The following Patents relate to specific peptides**

US 5,962,639

Peptides synthesised to match the human  $\alpha 1(I)$  and  $\alpha 2(I)$  telopeptide sequences of the type I collagen metabolites, preferably selected from among:

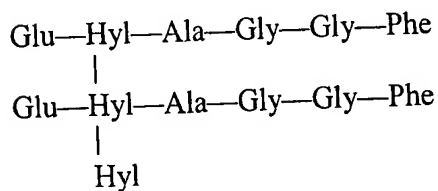
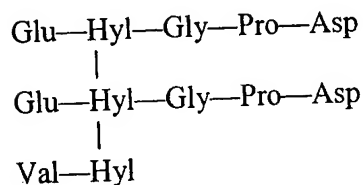
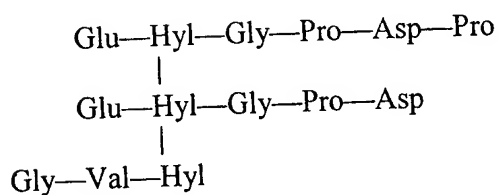
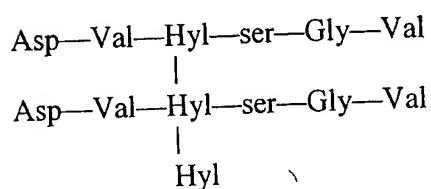
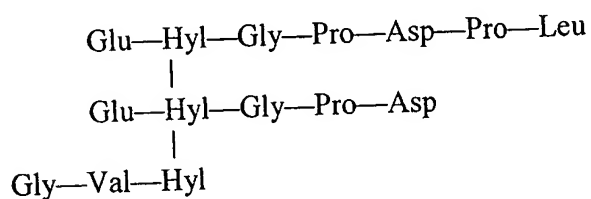
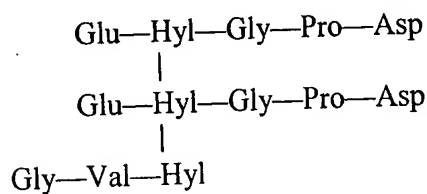
Asp-Glu-Lys-Ser-Thr-Gly-Gly;

Gln-Tyr-Asp-Gly-Lys-Gly-Val-Gly; and

Glu-Lys-Ala-His-Asp-Gly-Gly-Arg.

These peptides are useful as calibrators and antigens in immunoassays for detecting type I collagen degradation products from body fluids.

US 5,140,103



Wherein Hyl-Hyl-Hyl is hydroxylysyl pyridinoline.

US 5,750,647

Synthetic linear peptides embodied by Y-Tyr-Asp-Gly-X-Gly-Val-Gly which mimic the epitope recognised by mAb 1H11 (ATCC No. HB 10611) in crosslinked N-telopeptides of type I collagen (NTx).

US 5,817,755

Synthetic linear peptides embodied by Xaa-Tyr-Xaa-Gly-Xaa-Gly-Val-Gly which mimic the epitope recognised by mAb 1H11 (ATCC No. HB 10611) in cross-linked N-telopeptides of type I collagen (NTx).

Adamczyk et al.

The utility of two immunogens prepared from benzyl ester for development of assays for osteoporosis is disclosed

Atley et al.

An immunoassay for cross-linked N-telopeptides of type I collagen in urine or serum is used to show osteoclast mediated bone resorption.

Brame et al.

Extremely reactive -ketoaldehydes were identified as products of the isoprostane pathway. Their lysyl protein adducts were characterised. It was investigated whether isoprostane endoperoxide intermediates rearranged to levuglandin-like compounds.

Hanson et al

A molecular site specificity investigation of pyridinoline and pyrrole cross-links in Type I collagen of human bone.

Hughes et al

Proposes that amino-ketone forms of collagen cross-links undergo a spontaneous Knorr condensation with each other, or with other species to produce a pyrrolic cross-link.

Kemp et al

An investigation into Ehrlich chromogens. It is postulated that a structure is present in collagen azo-EC-peptides containing two EC groups shared between four peptide chains. Based on this structure about 15% of adult bone collagen contains EC groups.

Kuypers et al

Identification of the loci of collagen-associated Ehrlich chromogen in Type I collagen. The collagen-associated EC is postulated to be a trisubstituted pyrrole.

Lombard et al

Three reagents for detecting indole were compared. The reagents were Kovac, Ehrlich and p-dimethylaminocinnamaldehyde (DMCA). DMCA was found to be the most sensitive detector of indole. It also allowed detection of indole derivatives. Kovac reagent was the least sensitive.

McBrayer et al

An investigation into the diffusion coefficient and solubilities of several metals using the candidate metals as the electrode. Diffusion coefficients are estimated from a closed form solution of the diffusion equation.

Raghavan et al

An investigation of the diffusion of copper through dielectric films under bias temperature stress. The leakage current through various dielectric films was characterised as a function of electric field and elevated temperature. Both electric field and temperature were found to strongly affect the dielectric barrier lifetime.

Rajkumar et al

The reaction of levuglandin (LGE)<sub>2</sub> with proteins generates pyrrole derivatives that are detected with an Ehrlich assay. The pyrroles formed by the reaction of LGE<sub>2</sub> with simple amines are chemically sensitive, but a stable derivative is obtained by trifluoroacetylation.

Ramanakoppa et al

A report of the purification and characterisation of a glycation end-product derived from one of the major degradation products of ascorbate.

Salomon et al

A method of producing iso[4] LGE<sub>2</sub>-protein by radical-induced oxidation of arachidonic acid in the presence of protein or free radical-induced oxidation of LDL, the LGE<sub>2</sub> isomers may then be efficiently dequestered by covalent adduction with LDL-based amino acid groups.

LDL = low density lipoprotein



Scott et al

A method for isolating polypeptides associated with the Ehrlich chromogen from collagen digests utilising diazotized arylamine-cellulose supports. These peptides, in which the Ehrlich chromogen is "labelled" with yellow diazo colour constitute less than 0.5% of the collagen and have amino acid patterns similar to those around the cross link regions. The chromogen is not identical with pyridinoline also thought to be a polyvalent cross link.

This statement is being submitted before receipt of any office action on the merits. Thus, no fee is due for the filing of this paper. However, if a fee is due, please charge deposit account 50-0573.

Respectfully submitted,

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